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Preliminary communication

Antimalarial activities of new hydroxytrifluoromethylbenzylamines salts

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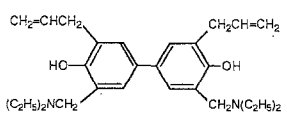
Summary — As a part of a research project on antimalarial agents, a series of hydroxytrifluoromethylbenzylamines and their hydrochloride and ammonium derivatives were prepared and evaluated as potential antimalarial agents. Some compounds were found to have a significant activity against *Plasmodium falciparum* FCC_2.

Résumé — Activités antimalariques de nouveaux dérivés d'hydroxytrifluorométhylbenzylamines. *Au cours du travail entrepris dans le but d'obtenir des composés à potentialité antimalarique, nous avons préparé une série de chlorhydrates et d'ammoniums dérivés d'hydroxytrifluorométhylbenzylamines et testé leurs activités potentielles. Certains de ces composés ont présenté une activité significative sur* P falciparum *FCC_2.*

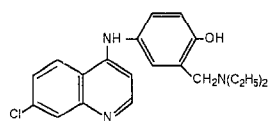
hydroxytrifluoromethylbenzylamine / antimalarial drug / Mannich reaction

Malaria is the most important of all infectious diseases. Worldwide, this disease affects hundreds of millions of people and over 2 millions die each year from malaria. This shocking reality is due largely to the emergence of drug resistant strains of *Plasmodium falciparum* [1–3].

Bialamicol and amodiaquine have been shown to possess significant and therapeutically useful biological activity as antimalarial drugs [4].



Bialamicol



Amodiaquine

The *o*-hydroxybenzylamine skeleton is an essential part of these active compounds. It has demonstrated the interest of CF_3 aromatic substitution in a series of 5-(aryloxy)-4-methylprimaquine analogues [5]. Also, mefloquine, which is the most promising of new antimalarial drugs in regions of chloroquine resistance, has CF_3 groups in its structure. This led us to synthesize hydroxytrifluoromethylbenzylamines and explore the antimalarial activities of their hydrochloride and ammonium derivatives.

Chemistry and pharmacology

The aminomethyl derivatives **1** were prepared in a Mannich reaction by reacting *m*-trifluoromethylphenol with secondary amines and formaldehyde in isopropanol [6].

The hydrochloride **2** and quaternary **3** salts were obtained by conventional procedures [7] by saturating the ethanolic solution of free bases with dry HCl gas and by heating under reflux amines in absolute ethanol with CH_3I (scheme 1).

The new compounds were identified by ^1H NMR analysis (tables I and II) and their purity established by controls on TLC and microanalysis. The ^{13}C NMR spectra and the ^{13}C–^{19}F coupling constants showed no *ortho*-substituents for the CF_3 group [8] and confirmed the structure in which aminomethyl group is in

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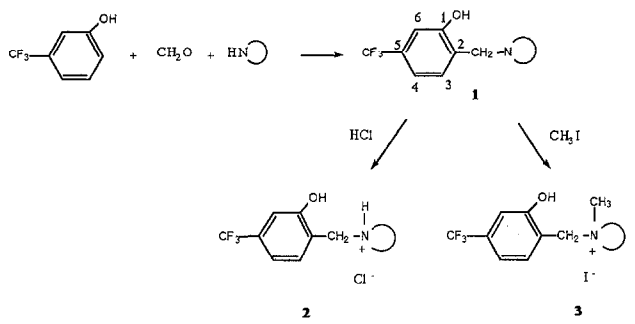
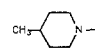
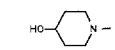
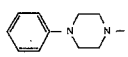
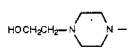


Scheme 1.

para position of the trifluoromethyl group. For example, the experimentally determined ^{13}C NMR spectrum (in D_2O) of the compound **2d** is:

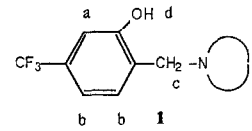
Chemical shifts (ppm)		^{13}C–^{19}F Coupling constants (Hz)
C1	158.6	–
C2	134.8	–
C3	122.2	–
C4	119.4	3.7
C5	136.1	37.0
C6	115.0	3.2

Table I. Yields of synthesis and melting points of hydroxy-trifluoromethylbenzylamines derivatives **1**, **2** and **3**.

		Yield (%)			mp °C		
		1	**2**	**3**	**1**[a]	**2**[b]	**3**[c]
a		93	75	88	oil	130-132	140-142
b		84	72	69	oil	134-136	152-154
c		90	99	86	35	170-172	144-146
d		98	89	84	108-110	164-166	150-152
e		67	74	96	122-124	166-168	150-152
f		79	83	88	40	162-164	170-172
g		92	85	86	oil	158-160	154-156
h		90	89	82	oil	154-156	134-136

Recrystallization from [a]absolute ethanol, [b]absolute ethanol-acetone (1:1), [c]ether.

Table II. ^1H NMR data of compounds **1**. Chemical shifts ($CDCl_3$/TMS) δ in ppm.



Compound N°	a	b	c	d
1a	7.06 (s,1H)	7.01 (m,2H)	3.67 (s,2H)	11.78 (s,1H)
1b	7.06 (s,1H)	7.01 (m,2H)	3.64 (s,2H)	11.49 (s,1H)
1c	7.06 (s,1H)	7.02 (m,2H)	3.75 (s,2H)	11.62 (s,1H)
1d	7.08 (s,1H)	7.03 (m,2H)	3.80 (s,2H)	11.68 (s,1H)
1e	7.08 (s,1H)	7.02 (m,2H)	3.68 (s,2H)	11.04 (s,1H)
1f	7.08 (s,1H)	7.02 (m,2H)	3.80 (s,2H)	11.12 (s,1H)
1g	7.10 (s,1H)	7.03 (m,2H)	3.72 (s,2H)	10.03 (s,1H)
1h	7.08 (s,1H)	7.00 (m,2H)	3.74 (s,2H)	11.61 (s,1H)

This structure is in concordance with the literature [9]: aminomethylation for variously substituted phenols always occurs at the position *ortho* to the hydroxy group, even if the *para* position is unoccupied.

These compounds were tested *in vitro* and *in vivo*. *In vitro* assay was carried out with a chloroquine sensitive FCC_2 strain. *In vivo* assay was conducted with female mice infected by *Plasmodium berghei* strain.

Results and discussion

The water-soluble derivatives were tested *in vitro* against human malaria parasite, *P falciparum* FCC_2. The *in vitro* test results (table III) indicate that the quaternary salts **3** are more effective than the hydrochloride **2**, except for hydroxypiperidine derivatives.

The antimalarial activity is greater for six than for five membered ring. With an oxygen atom in the six membered ring of morpholine nucleus, activity is reduced.

The superior antimalarial activity of compounds **3a**, **3b**, **3e** suggests that lipophilicity may play a role in determining the antimalarial activity of this series, although compounds **2c**, **2d** with hydroxy group are the most active of hydrochloride salts.

Compound **3b** was the most active of new derivatives and was selected only for testing in mice. Mice receiving 100 mg/kg per day had extended survival times (3 days) but no cures.

Table III. Antimalarial activities against *Plasmodium falciparum* of hydrochloride **2** and quaternary **3** salts.

N°	ED_{50}* (μg/ml)	N°	ED_{50}* (μg/ml)
2a	90±10	3a	4±0.5
2b	100±10	3b	0.3±0.2
2c	2.7±0.5	3c	9±1
2d	11±2	3d	32±4
2e	80±10	3e	4.1±0.5
2f	100±10	3f	100±10
2g	100±10	3g	20±3
2h	100±10	3h	20±3
chloroquine	0.025±0.005		

ED_{50} = effective dose 50 (concentration of a compound that inhibits 50% of *Plasmodium falciparum*, compared with the controls).

In conclusion, as product **3b** shows promising antimalarial activity, it would be particularly interesting to prepare other quaternary salts and explore the activity of new various alkylpiperidine derivatives.

Experimental protocols

Chemistry

Melting points were determined on a Kofler block and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Brucker 200 MHz instrument and chemical shifts are reported in δ units (ppm) relative to internal TMS. Microanalyses were within ± 0.4% of theoretical values and were performed on Perkin Elmer Microanalyser 240.

General procedures for preparation of Mannich bases ***1***

0.05 mol of amine was dissolved in 50 ml of isopropanol and 20 ml of 37% aqueous formaldehyde solution by heating 0.05 mol (8.10 g) of *m*-trifluoromethylphenol was added and the mixture was refluxed for 5 h. After standing at room temperature overnight and filtration, the solvent was removed *in vacuo* and the oily residue was purified by chromatography on silica gel using $CHCl_3$-acetone (7:3) as the eluent.

The hydrochloride salts **2** were prepared by dissolving compounds **1** in dry ethanol and ethanolic solution was saturated with dry HCl gas.

The quaternary salts **3** were obtained by heating under reflux for 4 h a mixture of 0.01 mol of compound **1** and 0.05 mol (7.10 g) of methyliodide in 50 ml of methanol. After cooling, the solvent was evaporated under diminished pressure. The solid was washed with ethanol and crystallized from ether.

Biology

In vitro screenings were carried out with a chloroquine sensitive FCC_2 strain of *Plasmodium falciparum*. This strain is maintained in our laboratory in continuous culture according to Trager and Jensen's methods [10] and kept in a CO_2 incubator. *In vivo* tests were conducted with *Plasmodium berghei* strain (Specia strain).

In vitro *assay*

Experiments were conducted in 24 plastic microtiter plates (Nunc). Erythrocyte suspension having a 0.2 to 0.3% parasitemia was added to RPMI 1640 (Gibco) containing serum and various concentrations of compounds. For each concentration, the assays were performed in quadruplate. Control culture was also introduced in the test. Stock solutions were prepared by dissolving compounds in distilled water and sterilised by passing through 0.2 μm filter. Required concentrations were freshly prepared by dilution of these solutions with culture medium. The cultures were maintained in CO_2 incubator at 37°C for 48 h [11]. After this time, final parasitemia were determined by counting the number of parasites per 3000 erythrocytes, in Giemsa-stained blood films.

In vivo *assay*

Female mice (OF_1-IFFA Credo), weighing about 25 g were used in our experiments. They were infected with *Plasmodium berghei* by intraperitoneal injection of 50 μl blood containing 2 10^7 infected erythrocytes. Ten mices were treated for each compound. Treatment started 1 h after injection and carried out for 5 days. It was administred orally as a singly dose of 100 mg/kg to each mouse. Experiment included a group of 10 animals infected and no treated and a group of 10 animals which were only treated. Parasitemia was estimated from Giemsa-stained tail blood film every day until the end of the experiment. Experimental compounds were dissolved in distilled water and given to animals. The infected untreated control mice died on either day 7 or 9.

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